REDUCING SEQUENCING REDUNDANCY THROUGH PRE-ORDERING OF M13 CLONES USING *Dde*I FINGERPRINTING

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Random selection of M13 clones for DNA sequencing is a common practice among sequencing laboratories. We are developing a partial digest fingerprinting technique that will allow us to minimize the number of M13 plaques needed to obtain adequate coverage of a region of DNA. Many different techniques have been suggested to pre-order clones prior to sequencing, and any such technique should be both fast enough and cheap enough to justify its use. Partial digest fingerprints provide the potential for generating restriction maps that define the orientation and degree of overlap among M13 clones. We will present results from a proof of principle experiment using random M13 clones with an average size of 1000 bp from a previously sequenced and assembled cosmid sub-library. Insert DNA was prepared by PCR using a fluorescence-labeled primer. After DNA quantitation, each sample was cut with *Dde*I using two different partialing conditions. Reaction products were analyzed on agarose using a Perkin-Elmer/Applied Biosystems (ABI) 362 Genescanner. Uncut DNA from each clone was also analyzed to determine insert size and identify any smaller PCR products generated by PCR infidelity. Ordered *Dde*I fragments obtained using this technique were identified and their sizes determined using ABI-supplied Genescanner software. Preliminary experiments show that this system can easily detect strongly overlapping clones, and has the potential of identifying a near-minimal spanning set of overlapping M13 clones. We will discuss newly-developed software for automated assembly of ordered fragments.

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